

Comparison of the ability of the three endogenous GnRHs to stimulate release of follicle-stimulating hormone and luteinizing hormone in chickens

John A. Proudman^{a,*}, Colin G. Scanes^{b,1}, Sara A. Johannsen^b,
Luc R. Berghman^c, Mary J. Camp^d

^a *Biotechnology and Germplasm Laboratory, Agricultural Research Service,
U.S. Department of Agriculture, Beltsville, MD 20705, USA*

^b *Department of Animal Science, Iowa State University, Ames, IA 50011, USA*

^c *Departments of Poultry Science and Veterinary Pathobiology,
Texas A&M University, College Station, TX 77843, USA*

^d *Biometrical Consulting Service, Agricultural Research Service,
U.S. Department of Agriculture, Beltsville, MD 20705, USA*

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Abstract

It is well established that GnRH can stimulate the release of LH and FSH in mammals. Two GnRHs have been found in the chicken hypothalamus, cGnRH-I and -II. There is controversy as to whether either peptide can stimulate release of FSH in birds. The present studies compared the ability of cGnRH-I and -II to stimulate the release of FSH and LH in chickens. Lamprey (l) GnRH-III may be a specific-releasing factor for FSH, as it selectively stimulates FSH release in rodents and cattle, and has been detected in the hypothalamus of rodents, sparrows and chickens. Therefore, the ability of lGnRH-III to stimulate LH and FSH release was also examined. In our first experiment, the effects of cGnRH-I and -II were studied using 17-week prepubertal females. Intravenous injection of cGnRH-II at 1 and 10 µg/kg BW significantly increased LH secretion more than did cGnRH-I. Neither peptide significantly increased plasma FSH levels. In our second study, we administered cGnRH-I, -II or lGnRH-III to mature males maintained on a short photoperiod. cGnRH-II was again more potent than cGnRH-I in stimulating LH release, while lGnRH-III produced a modest LH rise. No GnRH

* Corresponding author. Tel.: +1 301 504 8094; fax: +1 301 504 5123.

E-mail address: JohnP@anri.barc.usda.gov (J.A. Proudman).

¹ Present address: Office of Research/Department of Poultry Science, Mississippi State University, Mississippi, MS 39762, USA.

peptide provided specific or potent stimulus to FSH secretion, although the high dose of cGnRH-II modestly enhanced FSH levels in the adult male ($P < 0.05$). Our results are not consistent with the view that lGnRH-III is a specific FSH-releasing hormone across multiple classes of vertebrates. We conclude that the mechanism by which independent release of FSH occurs in chickens remains unresolved.

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Keywords: Gonadotropin-releasing hormone; Chicken; FSH; LH; LHRH

1. Introduction

It is well established that gonadotropin-releasing hormone (GnRH) can stimulate the release of both luteinizing hormone (LH) and follicle stimulating hormone (FSH) in mammals (see reviews [1–3]). There are at least two decapeptide-releasing hormones for LH in the chicken hypothalamus; namely chicken (c) GnRH-I and -II [4–6]. The discovery of a second gonadotropin-releasing factor in chickens led to speculation that cGnRH-II might be predominantly an FSH-releasing factor [6], but there is controversy as both to whether cGnRH-I and/or cGnRH-II can stimulate release of FSH in birds and whether cGnRH-II has a physiological role in the control of even LH. Mammalian GnRH and both cGnRH-I and -II have been reported to stimulate the release of LH and FSH from rat pituitary cells in vitro [6] and from quail pituitary tissue in vitro and in vivo [7,8]. Similarly, both cGnRH-I and -II stimulate FSH release from chicken pituitary cells in vitro [9]. However, no effect of mammalian GnRH on FSH release was seen with turkey pituitary tissue in vitro [10] and only a modest response was observed in vivo [11]. Several studies have shown little or no FSH response to cGnRH-I challenges in vivo in non-photostimulated immature intact [12,13] or ovariectomized [13] chickens and in young cockerels [14]. The present studies compare the ability of cGnRH-I and -II to stimulate the release of FSH and LH in intact chickens using models where circulating LH and FSH secretion are likely to be basal.

Lamprey GnRH-III (lGnRH-III) has been proposed as the releasing factor for FSH in view of the selective increase of FSH release evoked in rodents ([15–17] and reviews [18,19]) and in cattle [20]. Moreover, lGnRH-III has been detected in both the rodent [21,22] and avian hypothalamus [23]. Therefore, the ability of lGnRH-III to stimulate LH and FSH release in adult chickens is also examined.

2. Materials and methods

2.1. Experiment 1

2.1.1. Animals

The effects of cGnRH-I and -II on FSH and LH release in vivo were examined with 17-week White Leghorn pullets housed singly in cages. The birds were housed in an

open-sided building in July, and therefore, received a stimulatory photoperiod. The birds had free access to feed (a commercial diet) and water.

2.1.2. Treatment and blood sampling

Peptides (cGnRH-I and/or -II, Peninsula Laboratories, San Carlos, CA) were administered by the brachial vein as a single injection or as sequential injections 30 min apart. Blood samples were taken by venipuncture from the contra-lateral brachial vein. Peptides were injected at doses of 1 or 10 $\mu\text{g/kg}$ BW based on earlier studies [24]. Two protocols were used. In the first, the effectiveness of each peptide in stimulating secretion of LH and FSH was studied at each of the two doses. Each peptide/dose combination was administered to six different birds and blood samples were collected immediately prior to injection and 2.5, 5, 10, 20 and 40 min after injection. The second sampling protocol was designed to determine whether any effects of cGnRH-I and -II were additive. One group ($n=6$) received the high dose (10 $\mu\text{g/kg}$ BW) of both peptides in a single bolus, followed by collection of blood samples at 2.5, 5, 10, 20, 30, 40, 50 and 70 min. A second group ($n=6$) received 10 $\mu\text{g/kg}$ BW of cGnRH-II at 0 min, and then received the same dose of cGnRH-I immediately following collection of the 30-min sample.

2.1.3. Statistical analysis

The hormone secretion data were analyzed using the area under the curve over the appropriate time period. The initial pre-injection value for each replicate was taken as the baseline and all subsequent values were adjusted to that baseline. The trapezoid method was used to calculate the area under the curve, and the area values were analyzed for the variables FSH and LH. Values obtained from the administration of single peptides were analyzed as two-factor linear models using Proc Mixed [25] with hormone and dose as the factors. The assumptions of the general linear model were tested and met. The data from the second sampling protocol were analyzed for the first 30 min (response to first injection), the next 40 min (response to second injection), and for the total time, using Proc *t*-test [25].

2.2. Experiment 2

2.2.1. Animals

This study employed seven (~ 32 -week-old) adult broiler breeder male chickens. These males had been used previously for breeding on a photoperiod on 14-h light:10-h dark. Birds were housed in cages and received feed (a commercial diet) and water ad libitum. Before experimentation (2 weeks prior to the first challenge), the daylength was reduced to 10-h light:14-h dark in an attempt to reduce basal circulating concentrations of FSH.

2.2.2. Treatment and blood sampling

Birds were challenged with injection into the brachial vein of vehicle (saline), cGnRH-I (1 or 10 $\mu\text{g/kg}$), cGnRH-II (1 or 10 $\mu\text{g/kg}$) or lGnRH-III (1 or 10 $\mu\text{g/kg}$) in a Latin square design with a weekly interval between challenges. Chicken GnRH-I and -II were purchased from Bachem (King of Prussia, PA). Lamprey GnRH-III was synthesized by solid-phase

methodology and was purified to more than 99% purity by HPLC in the Protein Facility of Louisiana State University. Blood samples (2 ml) were taken by venipuncture from the brachial vein 1 min prior and 5, 10 and 20 min after challenge.

2.2.3. Statistical analysis

The hormone secretion data were analyzed using the area under the curve values calculated by the trapezoid method. Values were analyzed as one-factor linear models using Proc Mixed, where Treatment was the factor [25]. The assumptions of the general linear model were tested. To correct for variance heterogeneity in FSH and LH, the variance grouping technique was used. This technique may be described as follows: Proc Mixed can partition the pooled experimental variance. When the data was normally distributed but the treatment variances were heterogenous, treatments with ‘small’ variances were grouped together for a separate estimate of the experimental variance. The same was done for treatments with ‘medium’ or ‘large’ variances. The resulting analysis is analogous to the *t*-test with unequal variances. Tests of hypotheses, such as means comparisons, were conducted and the standard errors were computed from the separate estimates of the experimental variances. Where treatment differences were significant, mean comparisons were done with Sidak adjusted *P*-values so that the experiment-wise error was 0.05.

2.3. Hormone analysis

Blood samples were collected in tubes containing heparin and plasma was stored at -20°C prior to determination of FSH and LH concentrations by homologous radioimmunoassay [14,26]. The intra- and inter-assay variability for these analyses were 3.6 and 6.6%, respectively, for FSH, and 0.2 and 9.4% for LH.

3. Results

3.1. Experiment 1

Fig. 1 illustrates the effects of cGnRH-I and -II on plasma concentrations of LH and FSH in pullets approaching sexual maturity. Changes in hormone secretion were compared using the area under the curve for each GnRH and dose, and the results of the analysis of variance and comparison of means is presented in Table 1. Injection of cGnRH-I or -II resulted in a near doubling of plasma LH concentration at 2.5 min after challenge (Fig. 1). The response to cGnRH was highly significant ($P = 0.0104$), but the response did not vary significantly with dose ($P > 0.05$). Chicken GnRH-II administration resulted in a significantly greater LH response than cGnRH-I ($P < 0.05$). There was no significant change in FSH secretion in response to GnRH challenge.

The hormone response to cGnRH-I and -II did not differ when the peptides were administered together versus 30 min apart (Fig. 2). Statistical comparison of the area under the curve revealed no differences between the two treatments in the amount of LH or FSH secreted over treatment periods 0–30, 30–70 or 0–70 min due to the administration regimen used for the peptides (Table 2).

Table 1

Analysis of variance and means comparisons of changes in FSH and LH secretion in immature (17 weeks) White Leghorn pullets challenged with cGnRH-I or cGnRH-II administered at doses of 1 or 10 $\mu\text{g/kg}$ BW ($n=6$)

Source	Analysis of variance				
	DF	FSH		LH	
		<i>F</i> -value	<i>P</i> -value	<i>F</i> -value	<i>P</i> -value
cGnRH	1	0.38	0.5446	7.99	0.0104*
Dose	1	0.03	0.8559	0.99	0.3327
cGnRH \times dose	1	0.19	0.6642	0.05	0.8281
Peptide	Means comparisons				
	FSH			LH	
	Dose		Hormone ave	Dose	
	1	10		1	10
cGnRH-I	18.67	22.95	20.81	40.54	59.47
cGnRH-II	36.30	25.88	31.09	104.41	134.13
Dose ave	27.49	24.42		72.48	96.80
					50.01b
					119.27a

The area under the curve was calculated for each individual with the initial value taken as the baseline and the area values were analyzed as described in Section 2. Hormone means with different letters (a and b) are significantly different at the 0.05 level.

* Significant treatment effect.

Table 2

Statistical comparison of changes in LH and FSH secretion in immature (17-week) White Leghorn pullets challenged with cGnRH-I and -II administered either concurrently or sequentially at a dose of 10 $\mu\text{g/kg}$ BW ($n=6$)

Variable	<i>t</i> -Test analysis					
	FSH			LH		
	DF	<i>t</i> -Value	<i>P</i> -value	DF	<i>t</i> -Value	<i>P</i> -value
T1	10	−0.59	0.5659	10	−0.19	0.8507
T2	8	−0.25	0.8059	9	−1.51	0.1647
Total	8	0.03	0.9775	9	−1.02	0.3341
Variable	Means					
	FSH			LH		
	GnRH-I + II	GnRH-II, I		GnRH-I + II	GnRH-II, I	
T1	42.04	68.27		159.87	170.95	
T2	39.59	49.84		69.00	185.54	
Total	87.95	85.96		228.87	353.29	

The area under the curve was calculated for each individual with the initial value taken as the baseline and the area values were analyzed for the first 30 min (0–30), T1, the next 40 min (30–70), T2 and the total time, Total, using Proc *t*-test [25]. As none of the effects were statistically significant, mean comparisons were not done.

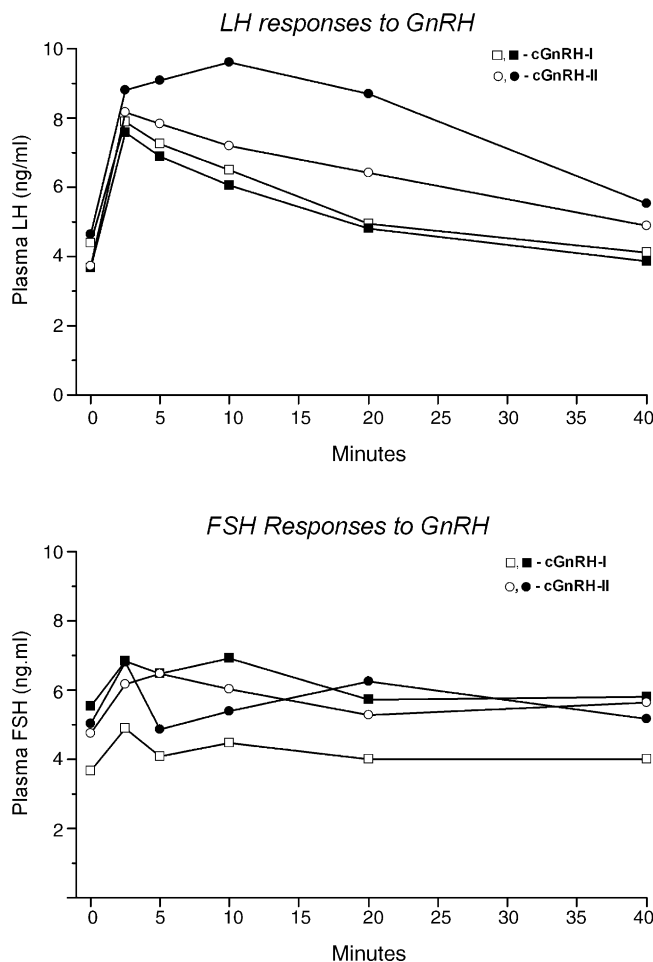


Fig. 1. Mean plasma LH and FSH concentrations of 17-week White Leghorn pullets injected with cGnRH-I or -II at doses of 1 µg (open symbols) or 10 µg (closed symbols)/kg BW ($n=6$). Statistical analysis is presented in Table 1.

3.2. Experiment 2

Fig. 3 summarizes the effects of cGnRH-I, -II and lGnRH-III on plasma concentrations of LH and FSH in adult male chickens. Analysis of variance revealed a highly significant effect of treatment on release of both hormones (Table 3).

Plasma LH secretion was significantly increased ($P < 0.05$) by cGnRH-I (1 and 10 µg/kg BW) and cGnRH-II (10 µg/kg BW). The latter treatment resulted in a two-fold greater LH response than any other treatment, and appeared to increase LH secretion in a dose-dependent manner. Lamprey GnRH-III administered at the highest dose resulted in an LH response that was intermediate to all the other treatments. Plasma concentrations

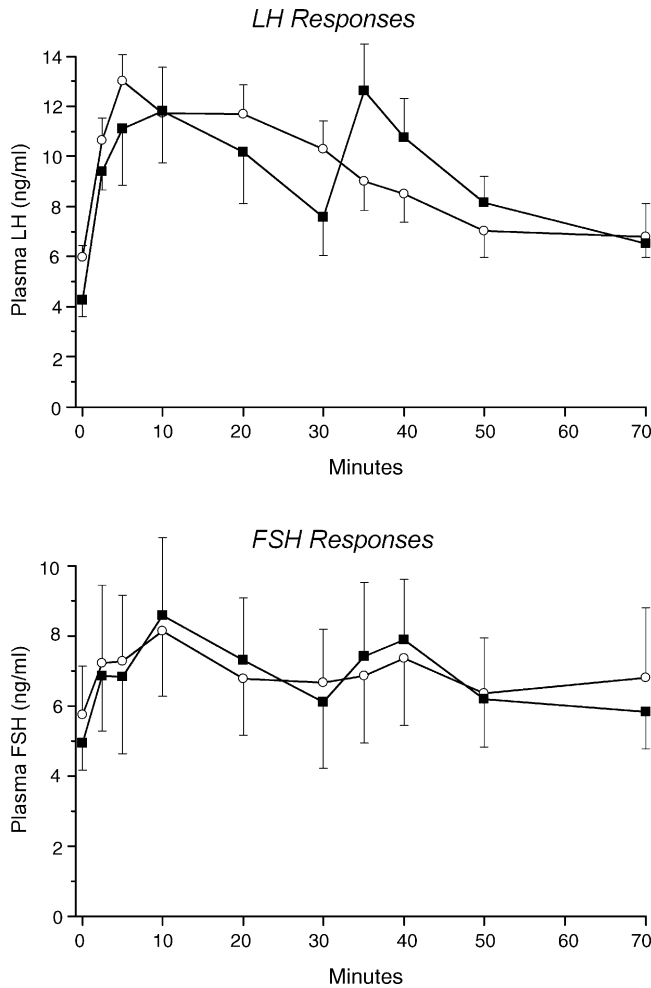


Fig. 2. Comparison of the effects of concurrent or sequential administration of cGnRH-I and -II on plasma levels of FSH and LH (mean \pm S.E.M.). White leghorn pullets (17 weeks; $n=6$) were administered 10 $\mu\text{g/kg}$ BW of both cGnRH-I and -II (○) at 0 min. A similar group ($n=6$) was administered 10 $\mu\text{g/kg}$ BW of cGnRH-II at 0 min and 10 $\mu\text{g/kg}$ BW of cGnRH-I at 30 min (■). Statistical analysis is presented in Table 2.

of FSH were significantly increased only by cGnRH-II ($P<0.05$). Saline-injected animals showed no significant change in plasma LH or FSH levels during the sampling period.

4. Discussion

The regulation of gonadotropin secretion is complex. In birds, two neuroendocrine systems are pivotal in regulating the reproductive cycle: the GnRH/gonadotropin system and

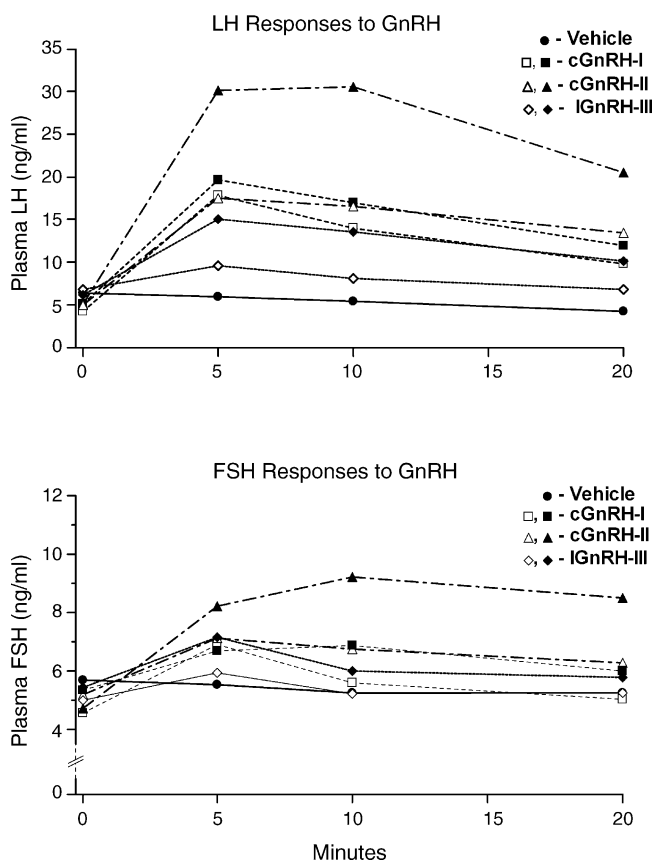


Fig. 3. Mean plasma LH and FSH concentrations of adult broiler breeder males injected with cGnRH-I or -II, or IGnRH-III, at doses of 1 μ g (open symbols) or 10 μ g (closed symbols)/kg BW ($n=7$). Statistical analysis is presented in Table 3.

the VIP/prolactin system (see [27] for review). The role(s) of the hypothalamic GnRH peptides in the differential regulation of LH and FSH secretion remains particularly controversial in all species. The original decapeptide isolated and sequenced from porcine and ovine hypothalami, mammalian GnRH, stimulates LH release from all species studied and, in mammals, will simultaneously stimulate LH and FSH secretion when injected as an intravenous bolus (see [28] for review). However, most vertebrates are now known to possess two or three GnRH molecules, one of which (cGnRH-II) is found ubiquitously across species and tissues. In this study, we directly compared, for the first time, the gonadotropin-releasing effects of bolus injections of three GnRH peptides in the chicken, a species distinguished by both a daily ovulatory cycle and an apparently complete separation of the two gonadotropins, LH and FSH, into separate pituitary cell populations [29,30]. Our results have demonstrated that the three GnRH peptides have clear but distinctly different abilities to stimulate LH secretion under the conditions studied, and that none of these peptides either specifically or robustly stimulates the secretion of chicken FSH.

Table 3

Analysis of variance and means comparisons of changes in FSH and LH secretion in male chickens challenged with cGnRH-I or -II, or lGnRH-III, administered at doses of 1 or 10 $\mu\text{g/kg}$ BW ($n = 7$)

Treatment	Analysis of variance	
FSH		
DF	6	
F-value	5.62	
P-value	0.0005	
LH		
DF	6	
F-value	17.49	
P-value	<0.0001	
Treatment	Means and means comparisons	
	FSH	LH
Vehicle	~6.09b	~19.56c
cGnRH-I, 1 $\mu\text{g/kg}$	21.90b	168.40ab
cGnRH-I, 10 $\mu\text{g/kg}$	21.59b	196.44ab
cGnRH-II, 1 $\mu\text{g/kg}$	26.94ab	191.20abc
cGnRH-II, 10 $\mu\text{g/kg}$	69.77a	393.63a
lGnRH-III, 1 $\mu\text{g/kg}$	7.48b	22.99bc
lGnRH-III, 10 $\mu\text{g/kg}$	11.02b	118.34abc

The area under the curve was calculated for each individual and the area values were analyzed as described in Section 2. Means with different letters (a–c) are different at the 0.05 significance level.

4.1. GnRH stimulation of LH secretion

We found that cGnRH-II was more potent than cGnRH-I in stimulating LH release in vivo in both immature pullets and adult roosters. This result is similar to those reported in several in vitro studies (chickens [9,31]; turkeys [32]). Previous in vivo studies have variously reported cGnRH-I and -II to have similar activities in stimulating LH release (e.g. [31]) or have found that cGnRH-II [33] or cGnRH-I is more potent [34]. Our finding is supported by recent studies showing that the chicken GnRH receptor has approximately 10-fold greater affinity for cGnRH-II than for cGnRH-I [35] and that cGnRH-II is pre-configured in a bioactive conformation for binding to non-mammalian GnRH receptors, and therefore, has a high affinity for all GnRH receptors [36]. Despite its lower potency in stimulating LH release, there is strong evidence that cGnRH-I is involved in the physiological control of LH release in the chicken. This evidence includes the following: (1) cGnRH-I is present at substantial levels in the avian median eminence [37,38]; (2) concentrations of cGnRH-I change with reproductive state [39–41]; (3) cGnRH-I is released from the median eminence in response to stimulation with potassium [37,38]; and (4) active immunization of laying hens against cGnRH-I (but not cGnRH-II) results in complete regression of the reproductive season and depression of plasma LH [42]. The evidence for a role of cGnRH-II in the physiological control of LH release is less compelling. There is cGnRH-II present in the chicken median eminence [37,38], albeit at very low concentrations in some studies [38] but higher levels in others [43]. No release detectable by radioimmunoassay has been reported for cGnRH-II from avian median eminence (e.g. [38]). There are, however, changes in

hypothalamic nuclei content of GnRH-II with reproductive state in some studies [40,41] but not others [39]. Studies with antagonists to mammalian GnRH have concluded that only a single class of receptors to cGnRH-I and GnRH-II exists in birds [9], and prior studies [8] have shown no additive effect of concurrent cGnRH-I and -II administration on in vitro LH secretion by chicken pituitary cells [44] or either LH or FSH secretion by quail pituitary cells [8]. Our study comparing combined vs. sequential injection of cGnRH-I and -II in vivo supports this conclusion. In mammals, it is unlikely that GnRH-II (acting via the GnRH-II receptor) is the endogenous regulator of LH release from the gonadotroph [45].

4.2. GnRH stimulation of FSH secretion

Our results show that cGnRH-II, when administered at the highest dose, produces a significant increase in plasma FSH concentrations in adult male chickens. However, this 1.9-fold increase in plasma FSH was markedly less than the 6-fold increase in LH secretion that occurred concurrently. Chicken GnRH-I had no effect on FSH levels in males, and neither peptide increased FSH levels in immature pullets. These results are consistent with other studies in the chicken, where cGnRH-I has failed to stimulate FSH secretion in intact chickens of varying age and sex [12–14]. The in vivo effect of cGnRH-II on FSH secretion in the chicken has not, to our knowledge, been reported previously. These results stand in contrast to mammalian studies, where GnRH will stimulate secretion of both LH and FSH (for review, see [1–3,28]). The differential regulation of LH and FSH synthesis and secretion in mammals appears to be achieved mainly by the pulsatile pattern of GnRH release (for review, see [46]). However, pulsatile administration of GnRH and various combined treatments with activin, follistatin and estradiol have not been able to produce the singular pulses of FSH observed occasionally in mammals [28]. In adult male chickens, the pulsatile pattern of FSH secretion is largely independent of LH pulses, suggesting that LH and FSH secretion are independently regulated [47]. These and other observations continue to suggest the existence of a hypothalamic FSH-releasing factor, first proposed more than 40 years ago [48]. Recent studies have indicated that lGnRH-III may be a distinct releasing hormone for FSH in some species ([15–17,22] and reviews [18,19]), although an initial report of selective release of FSH in cattle [20] has recently been questioned [49]. This peptide is widely distributed in the central nervous system of two species of sparrow [23], and is reportedly present in the chicken hypothalamus [50]. Although Bentley et al. [23] found the distribution of lGnRH-III to be very different from that of cGnRH-I or -II in most brain areas, they did report proximity of immunoreactive lGnRH-III and cGnRH-I in some tissues and the presence of dense lGnRH-III fibers in the median eminence. Peripheral injection of lGnRH-III resulted in significant release of LH in non-breeding sparrows [23]. Our present data demonstrate no significant in vivo release of FSH in response to lGnRH-III challenge in adult male chickens, but lGnRH-III at a dose of 10 µg/kg resulted in a modest increase in plasma LH that was intermediate to that observed with cGnRH-I and -II. These observations are not consistent with the view that lGnRH-III is a specific FSH-releasing hormone across multiple classes of vertebrates. Our results clearly show that none of the GnRH peptides known to be present in the chicken produced either a robust or selective stimulation of FSH secretion under the conditions employed. It is possible that the absence of an FSH response in our study reflects the lack of priming doses of lGnRH-III [51]. Alternatively, the episodic

administration of GnRH at intervals approximating those observed for FSH and LH pulses in Vizcarra et al. [47] may provide a more effective stimulus to gonadotropin secretion than a bolus injection. Clearly, the mechanism by which independent pulsatile release of FSH occurs in chickens remains unresolved.

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